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Geographical and ecological distributions of frog hemiclones suggest occurrence of both 'General-Purpose Genotype' and 'Frozen Niche Variation' clones

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Abstract

Asexuals often occupy broad geographical and ecological ranges. Two models have been proposed to explain the ubiquity of asexuals: the General-Purpose Genotype (GPG) and the Frozen Niche Variation (FNV) model. According to these models, asexuals differ in their ecological niche width and may occupy narrow specialist niches or ubiquitous niches. A thousand water frogs from 37 different populations located in France in different habitats were studied, and two (hemi)clonal hybrid types were identified genetically, *Rana esculenta* and *R. grafi*. Altogether, 13 hemiclones were identified both in *R. grafi* and *R. esculenta*. Three of these were geographically and ecologically widely distributed, and usually very common in populations. In contrast, the remaining 10 hemiclones had small geographical ranges and were restricted to special habitat types, suggesting ecological niche specialization. The results suggest that in hybridogenetic water frogs GPG and FNV hemiclones coexist.

Key words: Water frogs – asexuals – hybridogenesis – niche width – distribution

Introduction

Asexuality is common (Halkett et al. 2005) and includes parthenogenetic, gynogenetic and hybridogenetic taxa that are often of hybrid origin (Schmidt 1993; Bullini 1994). Despite the lack of meiotic recombination, the accumulation of deleterious mutations and the associated low genetic variability, all together driving asexuals supposedly down a dead end road, asexuals seem to perform fairly well in nature, occupy broad geographical and ecological ranges (Lynch 1984; Milinski 1994; Forbes et al. 1997; Vrijenhoek 1998; Guex et al. 2002; Pound et al. 2004), and have been proved to reach a considerably high evolutionary age (Hedges et al. 1992; Quattro et al. 1992; Spolsky et al. 1992; Van Doninck et al. 2002; Halkett et al. 2005). These discrepancies have led to much debate about the reasons for differential performance in asexuals.

In several hybridization complexes with cyclical asexuality, like hybridogenetic complexes (Schmidt 1993), hybrid lineages are defined on the basis of their (hemi)clones. Such complexes often occupy a great diversity of habitats, the whole set of clones conferring an adaptation to a wide range of environmental conditions (Forbes et al. 1997; Semlitsch et al. 1997). Two models have been proposed to explain the distribution of hybrids: (i) the General-Purpose Genotype (GPG) model (Baker 1965; Schultz 1971, 1977; Parker et al. 1977), and (ii) the Frozen Niche Variation (FNV) model (Vrijenhoek 1979). Both models predict a hybrid advantage in a certain niche while the second model additionally implies clone or hemiclone competition and local adaptation (Vrijenhoek 1994; Semlitsch et al. 1997).

More precisely, the GPG model predicts that (i) the hybrid's genotype fits a broad ecological niche exhibiting a similar fitness level in both parental and intermediate niches, and (ii) the hybrid reveals a broad tolerance to temporal and spatial heterogeneity of the environment with low fitness variation across relevant physical, chemical and biotic gradients (Parker et al. 1977; Schultz 1977; Vrijenhoek 1994). Clonal diversity, as a result, declines and only the most generally adapted clone persists (Lynch 1984). In contrast, the FNV model predicts that, in the context of interclonal selection, each clone (or hemiclone) exhibits local adaptation and exploits a different narrow range of resources along the environmental gradient, and hence, occupies a narrow niche only (Vrijenhoek 1979, 1994).

Thus, when looking at the realized ecological niche in the wild (i.e. as resulting from natural selection), the GPG model predicts that one clone (one hybrid lineage) will be distributed in very different habitats with contrasting ecological characteristics (ubiquitous clone) while the FNV model predicts that each clone will be distributed only in similar habitats (specialist clone; Fig. 1). Moreover, it is expected that GPG clones are common in diverse populations and geographically widespread in relation to other clones. An FNV clone, in contrast, should be locally abundant dependent on the habitat type, but should generally be less common than GPG clones regarding all populations.

Hybridogenetic waterfrogs of the genus *Rana* (Amphibia, Anura) are good candidates to test these models and have been used for that purpose in several experimental studies (Semlitsch et al. 1997; Hotz et al. 1999). The utilization of this species complex to test the GPG and FNV models results from (i) the fact that hybrids are characterized by a hemiclonal genome, (ii) evidence of hybrid 'superiority' in various traits found in several experimental studies (Tunner and Nopp 1979; Semlitsch and Reyer 1992; Semlitsch 1993; Hotz et al. 1999), and (iii) differences found in the ecological characteristics of parental species and hybrids regarding habitat occupation (Pagano et al. 2001a,b; Holenweg-Peter et al. 2002) and ecological requirements (Schmeller et al. 2005; Voituron et al. 2005).

*These authors contributed equally to this study.

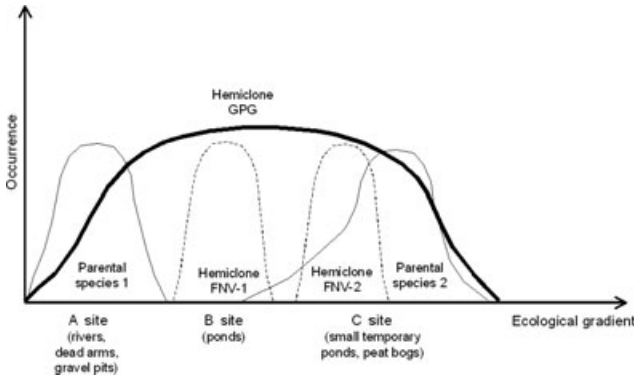

Fig. 1. General-Purpose Genotype (GPG) and Frozen Niche Variation (FNV) predictions. In the case of GPG, one genotype combination (one hemiclone) should be present in a broad range of distinct ecological habitats. FNV genotypes should be restricted only to certain habitats according to local adaptation and interclonal competition

Experimental studies, testing GPG and FNV predictions in hybridogenetic frogs, did not find support for the GPG model, but rather evidence for the FNV predictions (Semlitsch et al. 1996, 1997). Additional support for the FNV was found by Hotz et al. (1999), confirming interclonal selection and spontaneous heterosis as the basis for the ecological success of the hybrid lineage. All these experimental studies have in common that the predictions have been tested on hybrid tadpoles belonging to two neighbouring populations from Switzerland. Hence, the geographical and ecological scales were small. So far, no study has attempted at testing the predictions of the GPG and FNV models on (hemi)clones from a large ecological variety of habitat types and on a large geographical scale. In addition, experiments only examine the potential ecological niche of waterfrog tadpoles. In contrast, studying distribution of clones *in natura* allows evaluating the realized niche.

The present study aims at contributing to the understanding of the ecological success of hemiclones, as we assess the realized ecological niche on basis of a large sample of wild populations of two different hybridogenetic systems (LE and PG system) (Uzzell and Berger 1975) from habitats that differ strongly in several ecological parameters. Our data include detailed information on the geographical and ecological distribution of hemiclonal lineages and on their relative abundance. Therefore, we aim at assessing whether hybridogenetic water frogs confirm predictions of the GPG or the FNV model in regard to habitat utilization.

Materials and Methods

The hybridogenetic water frog complex in the study area

We studied 37 sites at the upper and lower Rhône valley, the Rhône Delta (Camargue), and along the Mediterranean coastline of southern France. Among the 37 sites ($n = 1025$ frogs), we focused on 28 populations containing hybrids ($n = 525$ frogs), of which 21 contained *Rana grafi*, Crochet et al., 1995 ($n = 451$ frogs) and seven *R. esculenta*, Linnaeus, 1758 ($n = 74$ frogs). Water frogs comprise two hybridogenetic systems in the studied area (Pagano et al. 2001a; Plötner and Schmeller 2001), in which *R. ridibunda* Pallas, 1771 (RR genome) is always implied as a parental species. In the LE system, the second parental species is *R. lessonae* Camerano, 1882 (LL genome), while in the PG system it is the Iberian species *R. perezi* Seoane, 1885 (PP genome) (Berger 1988, 1990).

Interspecies hybrids frequently occur in these waterfrogs, because of (i) hybrid fertility (Tunner and Heppich-Tunner 1991) and (ii) hybrid maintenance by matings with one of the parental species. The maintenance of the hybrid lineage by backcrosses occurs by hybridogenesis, a hemiclonal hybridization mechanism (Vrijenhoek et al. 1977). The characteristic feature of this reproductive mode is the elimination of one parental genome prior to meiosis during the hybrid's gametogenesis and the endoreduplication of the remaining chromosome set. As the hybridogens usually eliminate the none-R genome during gametogenesis and transmit the R-genome clonally, we find R-hemiclones in both hybridogenetic systems (Schmeller 2004).

Individual taxon and hemiclone identification was inferred from allozymic variations at four to eight loci (LE and PG systems respectively) that proved to be diagnostic between parental species and hybrids (Appendix 1). Each hemiclone may be detected by its combination of allozymic allele markers of the R-genome. Several studies have already identified different hemiclones using two or three loci (Uzzell and Berger 1975; Semlitsch et al. 1996; Pagano et al. 1997; Hotz et al. 1999). Hemiclones have been named with capital letters and indexed numbers, with E for *R. esculenta* and G for *R. grafi*. In our study, non-neutral markers such as allozymes (Schmidt et al. 1998) are more useful than neutral ones (e.g. DNA microsatellites), because we want to detect FNV or GPG clones resulting from natural selection. The hybridogenetic character of female *R. esculenta* and *R. grafi* has been shown by testing primary oocytes. All sampled hybrid females showed *R. ridibunda* allelic products only (data not shown), and hence, were of hybridogenetic character, transmitting their *R. ridibunda* genome clonally (for methodological details see Vrijenhoek 1972; Vrijenhoek et al. 1977, 1978; Schmeller et al. 2001).

Habitat types

Eight sites of our 37 (A1–A3, B1–B3, C1–C2) were studied in the French upper-Rhône floodplain inhabited by taxa of the LE system. A detailed ecological description of these 8 sites using 13 variables (Pagano et al. 2001b) showed that sites strongly differed in their ecological characteristics and provided evidence for a gradient of river influence and oxygen availability, thus allowing the distinction of three groups. The first group (A) was composed of dead arms frequently connected to the river, and sites very close to the river. These sites were characterized by frequent flood disturbance and high water oxygen concentration. The second group (B) was composed of oxbow lakes, and was defined by intermediate flood influence and oxygen concentrations. The third group (C) covered peat bogs, which were more distant from the river. The flood influence on these sites was negligible; the amount of organic matter was high, and the oxygen concentrations low (Appendix 1). The present and a previous experimental study (Plénet et al. 2000) also found evidence that habitat selection and/or utilization by waterfrogs may be influenced, among other variables, by oxygen availability in the water. Following categorization based on these 10 sites and performing a principal component analysis (data not shown) with all our sites, we additionally affiliated 10 habitats in the Rhône Delta, four along the Mediterranean coastline, five habitats along the lower Rhône valley, and two habitats in the upper Rhône valley to the three different habitat types (Appendix 1, Table 1). Eight further samples were collected on roads and were grouped by means of the different habitat types close by (Table 1).

Hemiclonal diversity

We used the diversity index H and its derived equitability index E_H (Shannon 1948) to describe the clonal diversity among hybrids. Each distinct hemiclone has been considered as a category constituting the hybrid population. Hence, if only one hemiclone was present, diversity was zero.

Niche width

The niche breadth for each hybrid species was characterized by the three environmental variables, salinity, acidity (pH) and relative oxygen content of the water (O_2). These were reduced to two dimensions by a principle component analysis, which can be represented as a biplot (Gabriel 1971). The niche breadth of a hemiclone was defined by drawing a minimum convex polygon (MCP) around the

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Table 1. Characteristics of sites and population composition. Given are the population number (No.), the sample size (N), the number of each water frog taxa, with the number in parenthesis being the percentage of the whole assemblage represented by the taxon, hemiclones and their total number in parenthesis (hemiclones), the total number of hemiclones (N_H), and the diversity indexes H (Shannon) and E_H (Equitability). Habitat type S refers to road catches with the type of the closest habitat type(s) indexed. The environmental variables are the habitat type (Ht), salinity (μS cm^{-1}), pH-value (pH), relative oxygen level (O_2%) and the amount of organic matter (%OM)

No.	*n*	*R. ridibunda*	*R. perezi*	*R. grafi*	Hemiclones	N_H	H	E_H	Ht	μS cm^{-1}	pH	O_2%	%OM
01	26		10 (38)	16 (62)	G_6 (16)	1	0	0	B	926	6.8	130	
02	30		9 (30)	21 (70)	G_6 (21)	1	0	0	B	642	6.0	120	
04	37		19 (51)	18 (49)	G_5 (17), G_6 (1)	2	0.215	0.310	B	1856	5.6	110	
05	17			17 (100)	G_6 (17)	1	0	0	B	1200	6.8	120	
07	26		10 (39)	16 (61)	G_1 (2), G_5 (14)	2	0.377	0.544	S_B				
08	28		8 (29)	20 (71)	G_5 (19), G_8 (1)	2	0.199	0.286	B	1150	5.3	159	4.9
09	25		13 (52)	12 (48)	G_5 (12)	1	0	0	C	4690	6.0	70	23.4
10	22	22 (100)				0			B	870	5.8	65	14.9
11	45		18 (40)	27 (60)	G_5 (27)	1	0	0	S_A				
12	15			15 (100)	G_4 (1), G_5 (10), G_8 (1)	3	0.720	0.655	S_B				
13	34		18 (53)	16 (47)	G_5 (15), G_7 (1)	1	0.234	0.337	B	1050	5.3	200	5.3
14	59		5 (8)	54 (92)	G_5 (53), G_{10} (1)	1	0.092	0.133	S_B				
15	18	5 (28)	2 (11)	8 (44)	G_4 (5), G_5 (3)	1	0.662	0.954	S_{AB}				
16	32	1 (3)	2 (6)	29 (91)	G_5 (25), G_9 (1)	1	0.150	0.216	B	394	5.9	140	5.5
17	27	2 (7)	3 (11)	22 (82)	G_5 (20), G_{11} (2)	1	0.305	0.439	C	5200	5.4	90	16.1
18	36		7 (19)	29 (78)	G_1 (1), G_5 (27), G_8 (1)	3	0.299	0.272	S_{BC}				
19	48	4 (8)	7 (14)	37 (76)	G_2 (1), G_4 (2), G_5 (32), G_8 (2)	4	0.539	0.389	S_{BC}				
20	24		5 (21)	19 (79)	G_4 (3), G_5 (15), G_8 (1)	3	0.633	0.576	C	450	5.3	30	17.2
21	27	8 (30)	6 (22)	7 (26)	G_3 (4), G_5 (3)	2	0.683	0.985	S_A				
22	27	1 (4)	1 (4)	25 (93)	G_4 (9), G_5 (16)	2	0.653	0.943	A	20000	5.7	130	6.8
23	35		10 (29)	25 (71)	G_5 (23), G_9 (1)	2	0.168	0.242	C	1500	5.3	130	84.7
24	24		6 (25)	18 (75)	G_5 (18)	1	0	0	B	1020	6.3	100	
26	27	27 (100)				0			A	630	5.3	180	
27	21	20 (95)				0			A	689	6.2	65	
28	28	28 (100)				0			A	584	6.5	111	
29	28	28 (100)				0			A	860	6.4	100	
31	29	29 (100)				0			A	356	6.2	82	
		R. ridibunda	*R. lessonae*	*R. esculenta*									
A1	18	18 (100)				0			A	302	8.2	93.8	0.7
A2	18	18 (100)				0			A	396	7.8	87	4.0
A3	27	27 (100)				0			A	170	9.7	113.4	2.0
B1	19	1 (5.3)	7 (36.8)	11 (57.9)	E_1 (11)	1	0	0	B	288	7.9	68.7	16.0
B2	36		7 (19.4)	29 (80.6)	E_1 (29)	1	0	0	B	270	8.1	126.6	14.0
B3	16		3 (18.8)	13 (81.2)	E_1 (13)	1	0	0	B	270	8.1	49.4	12.0
B4	33	29 (87.8)		4 (12.2)	E_1 (4)	1	0	0	B				
B5	31	25 (80.6)		6 (19.4)	E_1 (3), E_2 (3)	2	0.693	1.000	B				
C1	16		13 (81.2)	3 (18.8)	E_1 (3)	1	0	0	C	224	7.2	40.6	74.0
C2	16		8 (50)	8 (50)	E_1 (8)	1	0	0	C	409	7.1	39	88.8

sites: the size of the polygon therefore is directly related to the width of the ecological niche that a genotype occupies. A randomization test was used to test whether the niche breadth was narrower than expected by chance (Manly 1997). For each genotype, the vector denoting occupation of a site was randomized, and the MCP for the random occupation was calculated. This was done 999 times, and the p-value calculated as the number of times the area of the MCP was larger than the actual area of the MCP divided by 1000. Hence, a niche breadth is ecologically explained, if 95% of all cases are smaller than the actual niche breadth given by the MCP. A sampling error as explanation can be excluded in those cases. Clearly, genotypes which were found at none or one site could not be included in this analysis. If genotypes were found at two sites, the distance between the sites was used as the 'area'.

Results

All the frogs sampled in the upper Rhône floodplains belonged to *R. ridibunda*, *R. esculenta* and *R. lessonae*. In the lower Rhône valley *R. ridibunda* was found exclusively, whereas in the Rhône Delta and the Mediterranean coastline *R. perezi* and *R. grafi* were sympatric in most populations (Table 1). *Rana esculenta* and *R. lessonae*, respectively *R. grafi* and *R. perezi*, co-inhabited in B and C sites, constituting LE and PG population systems. *Rana esculenta* predominated in site B where it co-occurred with *R. lessonae*, which was true in most B sites for *R. grafi*, too. However, *R. grafi* also dominated three of the four C sites. Hybrids were not found in the most flooded sites (A group) in which only *R. ridibunda* was present, except one population close to the Grande Rhône and the Mediterranean Sea (Table 1).

In total we found 11 R-hemiclones in *R. grafi*, and two in *R. esculenta* (Table 2) that differ between the two taxa. In both cases, these taxa had several common hemiclones: G_5 (78.6%), G_6 (12.4%), E_1 (95.9%) plus more rare ones (< 10%). The ecological and geographical distribution of the different hemiclones varied. The G-hemiclones (hemiclones of *R. grafi*) could be split into two groups, (i) the common hemiclones G_5, G_6 and (ii) the rare ones (Fig. 2). Hemiclone G_5 is the most widely distributed, found in almost all the habitats over almost all the geographical range of *R. grafi* covered in this study. Along the Mediterranean coastline, it is gradually replaced by G_6, which differs from G_5 at two alleles (Table 2). Hemiclone G_8 was found in extreme habitats, as was hemiclone G_9 and

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Table 2. List of the hemiclones recorded. The allelic sequence of hemiclone refers to the loci *ck*, *α--gdh*, *ldh-b*, *mpr-2*, *pgm*, *ahh*, *gpi*, *6pgdh* for G-hemiclones; *aat-1*, *ldh-b*, *mpi*, *pgm-2* for E-hemiclones. Relative abundance has been calculated separately between all G-hemiclones and all E-hemiclones

Hemiclone name	Allelic sequence	Relative abundance (%)
G_1	bababace	0.7
G_2	babcaace	0.2
G_3	babcbacc	0.9
G_4	babcbace	4.5
G_5	babcbacf	78.6
G_6	babcbbcc	12.4
G_7	babcbbce	0.2
G_8	baccbace	1.4
G_9	bbbcaace	0.5
G_{10}	bbbcbace	0.2
G_{11}	babcbaca	0.5
E_1	eaad	95.9
E_2	-cad	4.1

especially hemiclone G_4 (Fig. 3a). All other hemiclones were restricted to single habitats or were registered in road samples close to the respective habitat type only. The situation looks similar regarding E-hemiclones (hemiclones of *R. esculenta*) with (i) the common hemiclone E_1 found in almost all the habitats (Fig. 3b), and (ii) a rarer one E_2 found in a single population and habitat type (Table 1).

The number of different hemiclones ranged from one to four in different populations (Table 1). We found two G-hemiclones in A sites, five G-hemiclones and two E-hemiclones in B sites, and five G-hemiclones and one E-hemiclone in C sites. The hemiclonal diversity was higher on roads (seven different hemiclones; Tables 1 and 3). Using the Shannon and equitability index values in the different habitat types, we did not observe significant differences in hemiclonal diversity in either system, except for a significantly higher diversity of G-hemiclones in road samples when compared with the diversity in B sites (Table 3).

There is no evidence for any reduction (size change) in niche breadth because of abundance (Table 4). For all the genotypes the niche breadth can be explained by random placement amongst populations. However, in hemiclone G_4 only 9% of the cases computed during the randomization procedure are larger than the original size (Table 4).

Discussion

Our analysis of the geographical distributions of water frog hemiclones in France showed strong differences in abundance and localization of hemiclones. Several are extremely rare, restricted to one or very few sites, while others are rather common and widely distributed. Regarding habitat types, these hemiclones occur in different environmental conditions, i.e. exhibit habitat niche widths that vary in size (Fig. 3) suggesting that their ability to cope with these different habitats should reflect local adaptation (FNV clones) or ubiquity (GPG clones) (Vrijenhoek 1994; Semlitsch et al. 1997).

The randomization procedure to check whether the observed niche breadths of the more common clones (6 over 13) are due to historical reasons (random placement regarding ecological characteristics of sites) or due to ecology (local adaptation to certain specific habitats) does not support that niche breadth is affected only by ecology. However, the niche size of clone G_4 at least suggests partly an ecological effect. We assume that the result is biased by a too small ecological sampling, influencing the niche area in our model. Despite these limitations, our model underlines that historical reasons may be invoked to explain the observed distribution of these clones. Some of the investigated clones may have not had the time to spread, as suggested by clones, present in low frequency in a few locations with different environmental characteristics.

However, as (i) environmental conditions strongly differ between sites, as (ii) FNV was evidenced by other authors (Semlitsch et al. 1996, 1997), and as (iii) variation in ecological condition is shown to structure water frog assemblages

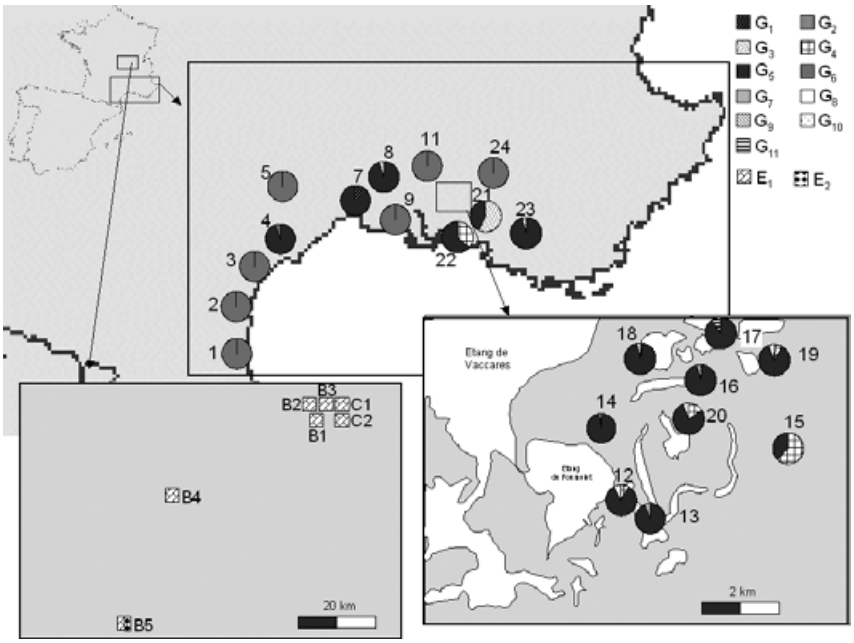

Fig. 2. Geographical distribution and population composition of the different hemiclones

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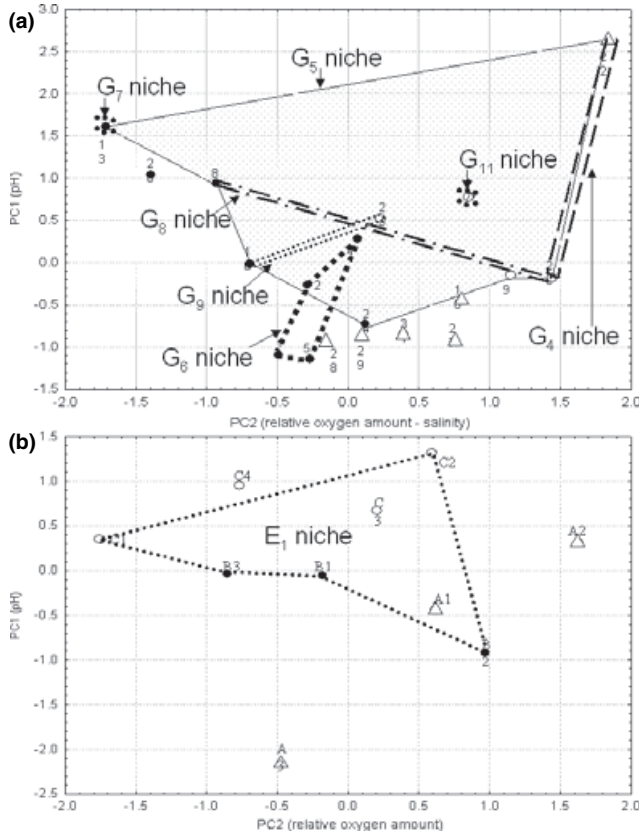

Fig. 3. Representation of ecological niche width (as areas) of different hemiclones [a: *R. grafi* hemiclones (G), PC1 = 53%, PC2 29%; b: *R. esculenta* hemiclones (E)], PC1 = 71%, PC2 19% regarding their occurrence in populations that differ in four ecological characteristics (oxygen, salinity, pH and organic matter). The figure is based on PCA coordinates of sites in respect to those four ecological variables. The General-Purpose Genotype hypothesis may be predicted when the ellipse comprises many populations of various habitat types. Frozen Niche Variation is predicted if ellipses are smaller or narrower. Triangles represent populations of the A habitat type, filled dots represent habitat type B and white dots habitat type C

Table 3. Summary of the hemiclonal diversity in the different habitat types (Ht). Shown are the number of populations per habitat type (N_{Ht}), the number of hybrids (N_h), the number of different hemiclones (N_H), the Shannon index H and the equitability E_H ± standard deviation. Below each hemiclonal group data the test statistics of the Mann–Whitney U-test (Z) are given with p indicating the level of significance

Ht	N_{Ht}	N_h	N_H	H	E_H
G					
A	1	25	2	0.65 ± 0.0	0.94 ± 0
B	8	155	5	0.10 ± 0.11	0.14 ± 0.16
C	4	78	5	0.28 ± 0.27	0.31 ± 0.25
S	8	186	7	0.42 ± 0.28	0.49 ± 0.36
			B × C	$Z = 1.19$	p > 0.05
			B × S	$Z = 1.99$	p = 0.046
			C × S	$Z = 0.76$	p > 0.05
E					
A	0	0	0	–	–
B	5	63	2	0.14 ± 0.31	0.20 ± 0.45
C	2	11	1	0	0
			B × C	$Z = -0.39$	p > 0.05

Table 4. Number of sites occupied and probability that niche breadth is narrower than random for genotypes of hemiclones of *Rana grafi* and *R. esculenta*

	R. grafi					*R. esculenta*
Genotype	G_4	G_5	G_6	G_8	G_9	E_1
Sites	2	10	4	2	2	5
p	0.09	0.18	0.88	0.28	0.77	0.67

(Negovetic et al. 2001; Pagano et al. 2001a,b; Holenweg-Peter et al. 2002; Plénet et al. 2005), adaptation to ecological conditions has to be considered likely. A more complete survey of ecological variables should allow us to specify niche breadth with more accuracy in order to increase statistical power of our model. In addition, we were not able to include the seven rarest hemiclones present at one site (suspected to be FNV) in the model. Hence, we cannot state statistically if they represent locally adapted clones or only recent ones. Finally, only experimental protocols such as reciprocal transplantations (Miaud and Merila 2001; Gomez-Mestre and Tejedo 2003) would allow us to determine if adaptation (GPG or FNV) occurs for such hemiclones.

Few of the detected hemiclones (G_5, G_6, E_1) have a wide ecological and geographical distribution and are dominant in almost all of the investigated populations. Historical reasons may certainly be invoked to explain the occurrence of these hemiclones, but these hemiclones (especially G_5) seem to compete well with any of the other hemiclones, as suggested by its dominance in almost all the investigated populations. Therefore, we argue that these widely distributed hemiclones should represent highly competitive GPGs. Most of the detected hemiclones have a narrow geographical and ecological distribution. Two reasons may account for such a distribution pattern, (i) recent origin due to primary hybridizations between the two parental species, or (ii) occupation of narrow ecological niches as assumed by the FNV model. Most of these hemiclones are found in the Rhône Delta, a region where *R. ridibunda* has also been shown to occur (Schmeller et al. 2005). As we lack data from subsequent years, we were unable to assess the persistence of different hemiclones, which clearly weakens our conclusions. Despite the lack of data, we believe the picture of the distribution of hemiclones drawn here is not an artefact from recent primary hybridizations of introduced *R. ridibunda*, as (i) subsequent primary hybridizations with either *R. perezi* or *R. lessonae* should have taken place many generations ago, (ii) *R. ridibunda* populations are rare, especially in southern France (Pagano et al. 2001a; Schmeller et al. 2005; Daf et al. 2006), (iii) the number of generations since introduction in the 1920s and 1950s (Pagano et al. 1997) should have been numerous enough to give opportunity for the origin and establishment of new hemiclones in various habitats and (iv) FNV has been shown in other study predictions (Semlitsch et al. 1996, 1997).

The differences between our results, suggesting the occurrence of GPGs, and previous experimental studies that support the FNV model only might result from the variety of habitats investigated. The experimental studies (Semlitsch et al. 1997; Hotz et al. 1999) were performed with frogs belonging to two neighbouring sites assignable to the habitat type C only (A. Pagano, pers. obs.). Those locations represent stable habitats that are expected to favour FNV hemiclones (Forbes et al. 1997; Semlitsch et al. 1997). In the present study, we

examined both diverse ecological conditions (three habitat types) and diverse geographical locations (from Southern to Eastern France). Our geographically extensive field investigation should make our study more reliable, when compared with the earlier experimental studies, in determining the realized niche of hemiclone characteristics.

Conclusions

More information is needed to understand which ecological variables are prominent in driving local adaptation in these natural hemiclonal hybrid populations. For a solid proof the more common clones should be used in reciprocal semi-natural experiments to test their ecological performances in comparison with rarer hemiclones. Disentangling the historical scenario and the influence of ecological variation in hemiclonal selection would provide interesting insights into the long-term persistence of such hybrid lineages and the stability of those hybridization systems. The respective rules of adaptive genetic differentiation, parental effects (Rasanen et al. 2003a,b) and/or phenotypic differentiation (Laugen et al. 2002) remain a major subject of debate in evolutionary biology. Hybridogenetic complexes in general and water frogs in particular represent a fascinating example of taxa combining sexual and asexual reproduction (Schmidt 1993). Those complexes appear to be suitable models to study genetic differentiation through interclonal selection and local adaptation in the light of asexuality's constraints (Vrijenhoek 1998). The ubiquity of hybrids found in the present and other studies (Günther 1990; Rybacki and Berger 1994) most likely refers to the combination of both GPG and FNV hemiclones. Therefore, our study also highlights that asexuality is not only penalized by deleterious mutations but may exhibit advantages such as broad and local adaptations, explaining the long-term persistence of such asexual hybrids (Kearney 2005).

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Résumé

Les distributions géographiques et écologiques des hémiclones de grenouilles suggèrent l'existence en parallèle du 'General Purpose Genotype' et de la 'Frozen Niche Variation'

Les lignées asexuelles occupent souvent de vastes distributions géographiques et écologiques. Deux modèles ont été proposés pour expliquer l'ubiquité des lignées asexuelles: le ≪ General-Purpose Genotype ≫ (GPG) et le ≪ Frozen Niche Variation ≫ (FNV). Ces deux modèles diffèrent dans leur prédiction quant à la largeur de niche occupée par les asexués. Les asexués occuperaient des niches étroites, pour les asexués spécialistes ou bien, des niches larges pour les ubiquistes. Un millier de grenouilles vertes provenant de 37 populations localisées en France dans différents habitats ont été étudiées et 2 types d'hybrides (hémi)clonaux ont été identifiés génétiquement, *R. esculenta* et *R. grafi*. Treize hémiclones ont été identifiés parmi ces 2 hybrides dont trois présentaient une vaste distribution géographique et écologique, et étaient commun dans les populations. Au contraire, les 10 autres hémiclones avaient des distributions géographiques localisées, et étaient restreints à certains types d'habitats, occupant des niches plutôt spécialisées. Ces résultats suggèrent la coexistence d'hémiclones FNV et GPG chez les grenouilles vertes hybridogénétiques.

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Appendix 1. Allozyme systems used to distinguish between the taxa. We used standard procedures of allozyme electrophoresis (Hebert and Beaton 1993) with several buffer systems with cellulose acetate (TG = Tris-Glycin, TB = Tris-Borat, TC = Tris-Citrat, TM = Tris-Maleat, PP = Phosphat; pH is given as decimal number) and a single one with starch gels (TC 6.0). The loci were reported to be highly polymorphic increasing the detection of the different hemiclones (Hotz and Uzzell 1982; Hotz 1983; Beerli 1994; Buckley et al. 1994). No. loci = number of scorable loci in the different buffer systems; diag. locus = locus used for identification; used in = in which system the locus was used (PG = *R. perezi*/*R. grafi*; LE = *R. lessonae*/*R. esculenta*)

Enzyme	Tissue	Buffer	No. loci	Diag. locus	Used in	EC no.
αgdh	Liver	TG 8.5	1		PG	1.1.1.8
6pgdh	Muscle	TB 8.9	1		PG	1.1.1.44
aat	Liver	TC 8.2	2	*aat-1*	LE	2.6.1.1
ahh	Liver	TG 8.5	1		PG	3.3.1.1
ck	Muscle	TG 8.5	1		PG	2.7.3.2
Ldh	Liver	TG 8.5	2	*ldh-b*	LE–PG	1.1.1.27
	Muscle	TC 6				
mpi	Liver	TM 7.0	1		LE	5.3.1.8
	Muscle	TC 6				
mpr	Muscle	TB 8.9	3	*mpr-2*	PG	
pgi	Liver	TG 8.5	1		PG	5.3.1.9
pgm	Muscle	TM 7.0	1	*pgm-2*	LE–PG	5.4.2.2
		TC 6				

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